

**Additional file 1 for:**

**Single-cell atlas of human urine-derived stem cell chondrogenesis establishes a non-invasive, xeno-free platform for translational cartilage research**

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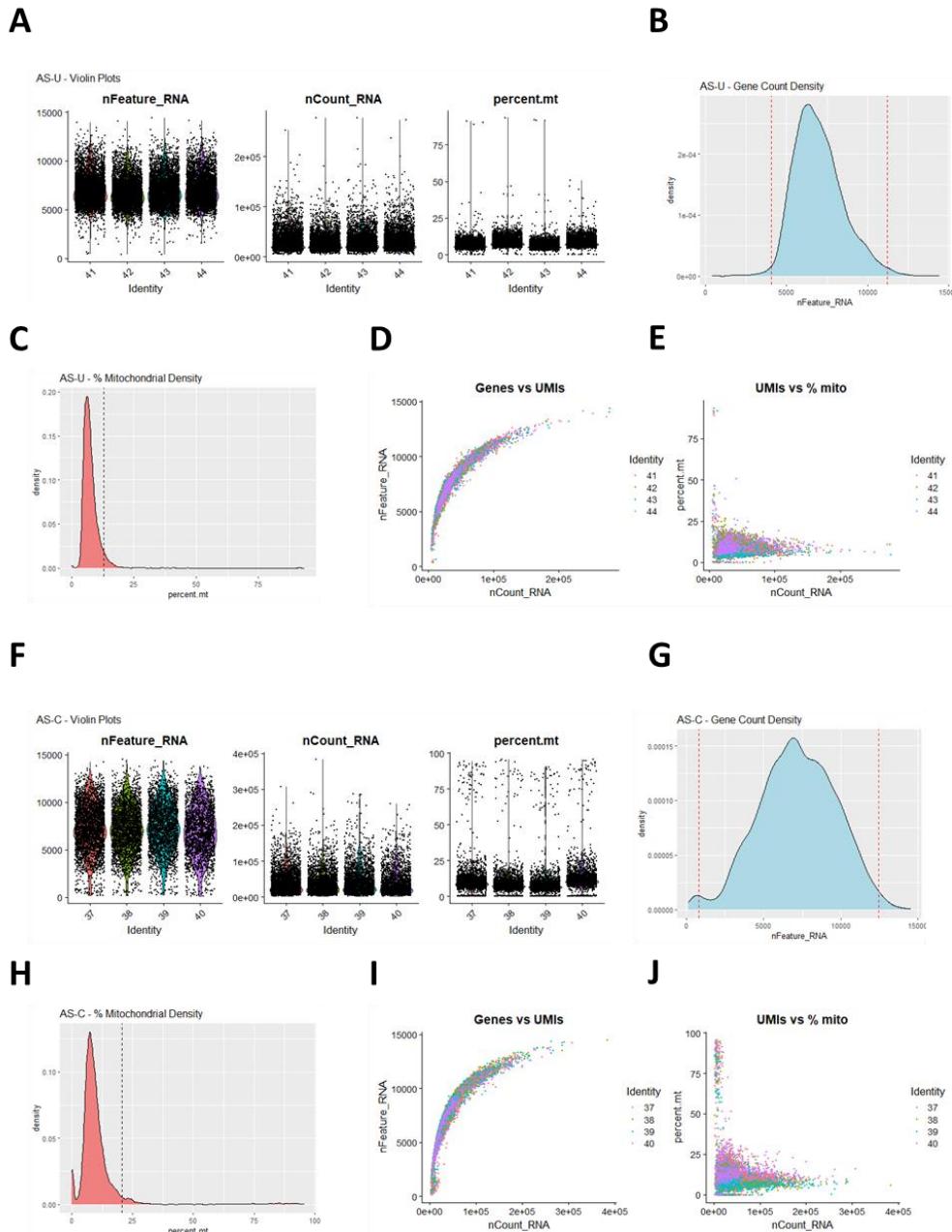
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**Keywords:**

single-cell RNA sequencing, urine-derived stem cells, chondrogenesis, xeno-free culture, cartilage biology, skeletal dysplasia modeling, translational regenerative medicine, disease modelling, tissue engineering, adult stem cells

## Supplementary Figures



**Figure S1: Quality control of single cell RNA-Seq data.**

Panels A–E (AS U= undifferentiated) and F–J (AS C= chondrogenic) show the same set of QC visualizations for each condition.

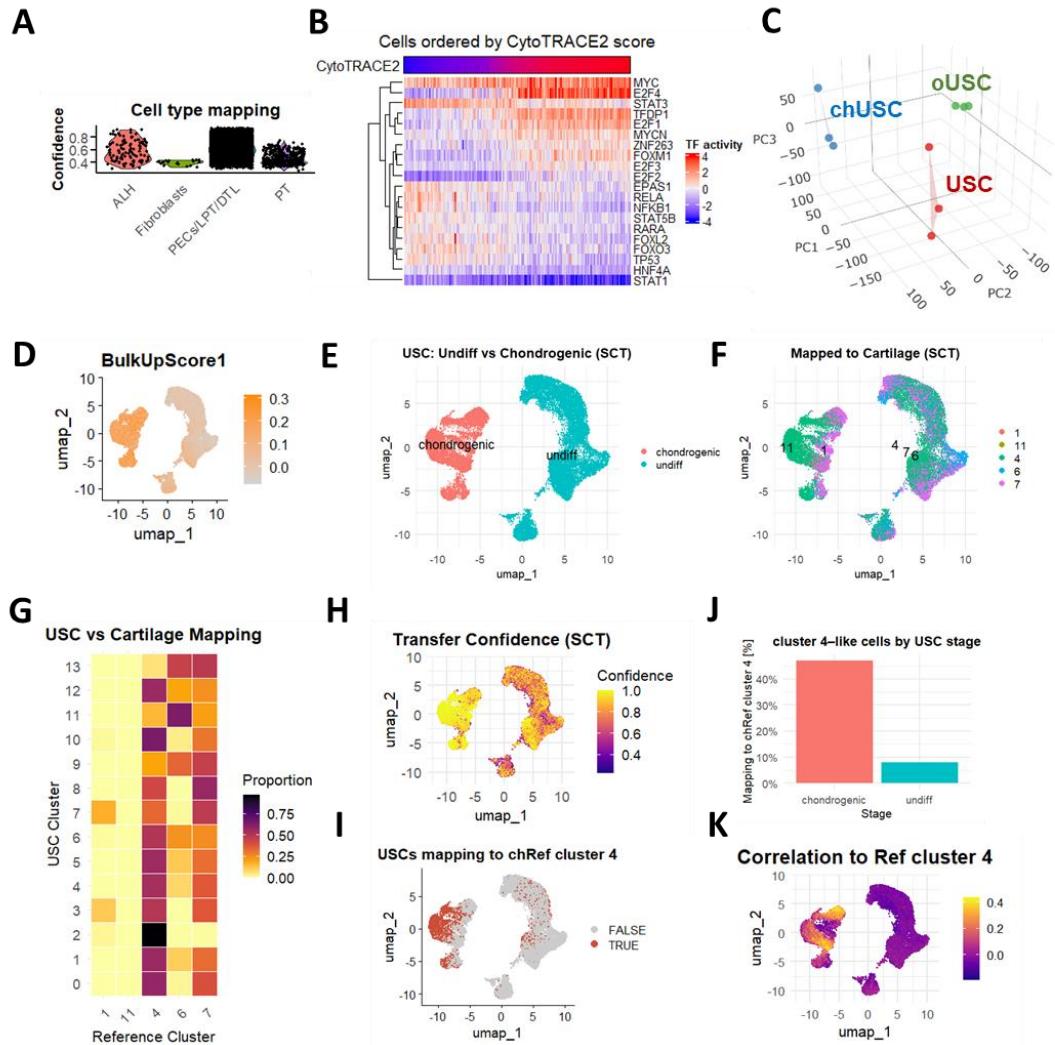
(A/F) Violin plots of detected genes per cell (nFeature\_RNA), UMI counts per cell (nCount\_RNA), and percent mitochondrial reads (percent.mt).

(B/G) Density of detected genes with dashed lines marking the 1st and 99th percentile thresholds.

(C/H) Density of percent mitochondrial reads with a dashed line at the 95th percentile threshold.

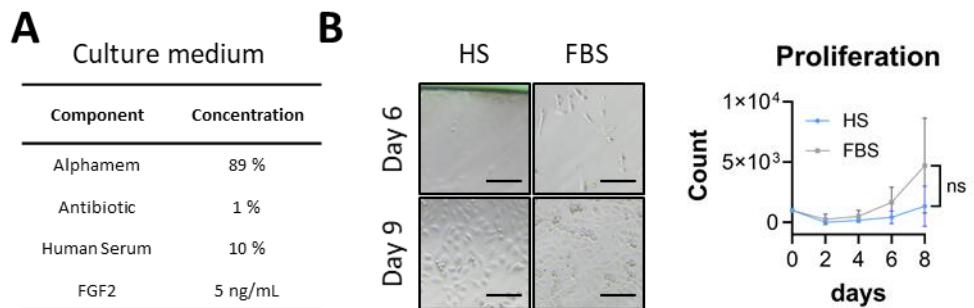
(D/I) Scatter of UMI counts vs. detected genes, illustrating library complexity.

(E/J) Scatter of UMI counts vs. percent mitochondrial reads, highlighting high mt cells.



**Figure S2. Supporting information for computational analysis.**

- (A) Violin plot showing predicted cell types for undifferentiated USCs.
- (B) Top 20 stemness-related transcription factor heatmap.
- (C) 3D-PCA of differentiated and undifferentiated replicates.
- (D) BulkUpScore reflecting upregulated genes in bulk RNA-sequencing, highlighted in the merged undifferentiated and chondrogenic USC Seurat object.
- (E) UMAP of the merged object with cell type annotations.
- (F) Merged UMAP mapped to a cartilage reference sample, highlighting predicted reference labels.
- (G) Heatmap visualizing mapping proportions of the merged USC object to the reference objects.
- (H) UMAP of the merged object with transfer-confidence color gradient.
- (I) UMAP of the merged object highlighting cells with mapping confidence to the chondrocyte reference cluster > 0.7.
- (J) Fraction of cells mapping to the reference cluster across stages.
- (K) Correlation values with the reference cluster mapped onto the merged UMAP.



**Figure S3. Expansion of USC under HS conditions.**

(A) Medium recipe for USC expansion using autologous serum.  
 (B) Representative images of colonies emerging under HS or FBS expansion at day 6 and day 9.  
 (C) Proliferation curves of HS- or FBS-expanded USCs ( $n = 3$ ).